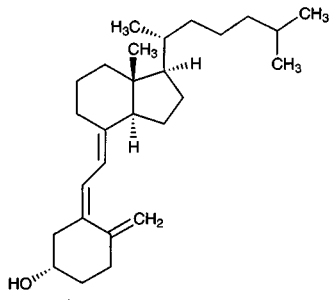
Vitamin D3



Molecular formula: $C_{27}H_{44}O$
Molecular weight: 384.65
CAS Registry No.: 67-97-0
Merck Index: 10157

SAMPLE

Matrix: blood

Sample preparation: 3-5 mL Plasma + 3 volumes ether, shake horizontally at 120 oscillations/min for 5 min, let stand for 1-2 min, freeze in dry ice/acetone, repeat ether extraction, extract aqueous layer with 4 volumes dichloromethane:MeOH 75:25, shake for 3-5 min, add 1 mL MeOH, shake for 15 s. Remove the organic layer and wash it twice with 100 mM pH 10.5 phosphate buffer, combine the dichloromethane and ether extracts and evaporate them to dryness under a stream of nitrogen, chromatograph on a 155 × 6 Sephadex LH-20 column with hexane:chloroform:MeOH 90:10:10, discard first 1 mL eluate, collect next 2.5 mL eluate. Evaporate to dryness under a stream of nitrogen, reconstitute with 500 µL hexane:chloroform 95:5 and chromatograph on a 145 × 6 Lipidex 5000 column (Packard) with hexane:chloroform 95:5, discard first 6 mL, collect next 4 mL. Evaporate to dryness under a stream of nitrogen, reconstitute the residue in 150 µL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.5 Zorbax Sil
Mobile phase: Dichloromethane:isopropanol 99.75:0.25
Flow rate: 2
Injection volume: 150
Detector: UV 254

CHROMATOGRAM

Retention time: 16

OTHER SUBSTANCES

Interfering: vitamin D2

KEY WORDS

plasma; normal phase; turkey; chicken; cow; pig; sheep; SPE

REFERENCE

Horst,R.L.; Littledike,E.T.; Riley,J.L.; Napoli,J.L. Quantitation of vitamin D and its metabolites and their plasma concentrations in five species of animals, *Anal.Biochem.*, **1981**, *116*, 189–203.

SAMPLE

Matrix: blood

Sample preparation: 2-3 mL Serum + 3.75 volumes hexane:isopropanol 1:2, shake for 30 min, let stand for 5 min, add 1.25 volume hexane, shake for 5 min, centrifuge at 600 g for 5 min, remove the upper organic layer, extract the aqueous layer twice with 1.25 volumes of hexane. Combine all the organic layers and evaporate them to dryness under a stream of nitrogen at 35°, reconstitute the residue in 50 µL hexane:isopropanol 80:20 and inject on to a 200 × 1 column of Kieselgel Si-60, Size A (Merck) and elute with hexane:isopropanol 80:20 at 2 mL/min, monitor at UV 254 and collect the vitamin D fraction at 6.0-7.5 min, evaporate the eluate to dryness under a stream of nitrogen. Reconstitute with 10 µL hexane:isopropanol 95:5 and inject on to a 50 × 4.6 Polygosil (Macherey-Nagel) + 250 × 4.6 7 µm Nucleosil 50-7 silica column, elute with hexane:isopropanol 85:5 at 2 mL/min, monitor the effluent at UV 254 and collect the vitamin D fraction at 4.5 min, evaporate the vitamin D fraction, reconstitute, inject an aliquot.

HPLC VARIABLES
Guard column: 30-40 μm pellicular C18 (Vydac)
Column: 250 × 4.6 5 μm TP C18 (Vydac)
Mobile phase: MeOH
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 6.0

OTHER SUBSTANCES
Extracted: vitamin D2

KEY WORDS
serum; normal phase; reverse phase

REFERENCE
Parviainen,M.T.; Savolainen,K.E.; Korhonen,P.H.; Alhava,E.M.; Visakorpi,J.K. An improved method for routine determination of vitamin D and its hydroxylated metabolites in serum from children and adults, *Clin.Chim.Acta*, **1981**, *114*, 233–247.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 2 mL EtOH, vortex for 3 min, add 3 mL hexane, vortex for 5 min, centrifuge at 1500 g for 10 min, repeat extraction with 3 mL hexane. Combine the hexane layers and wash with 2 mL MeOH:water 9:1. Remove the upper organic layer and filter (0.45 μm) it, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 33.4 μL EtOH, inject a 5 μL aliquot.

HPLC VARIABLES
Guard column: ODS
Column: 125 × 4 5 μm LiChrospher 100 RP-18 or 100 × 2.1 3 μm Spherisorb ODS-2
Mobile phase: Gradient. A was MeOH:water 99:1. B was MeOH:THF 70:30. A:B 100:0 for 2 min, 5:95 for 3.5 min, 0:100 for 4.5 min, re-equilibrate for 2 min (step gradients).
Flow rate: 1.5 (125 × 4 column) or 0.2 (100 × 2.1 column)
Injection volume: 5
Detector: UV 265

CHROMATOGRAM
Retention time: 4 (125 × 4 column), 4.5 (100 × 2.1 column)
Limit of detection: 0.44 ng (100 × 2.1 column), 19.8 ng (125 × 4 column)

OTHER SUBSTANCES
Extracted: retinyl palmitate (UV 328), vitamin E (α-tocopherol) (UV 284), vitamin K1 (UV 250)

KEY WORDS
cow; plasma; protect from light; degas stock solutions with helium; narrow bore

REFERENCE
Gomis,D.B.; Escotet Arias,V.J.; Fidalgo Alvarez,L.E.; Gutiérrez Alvarez,M.D. Simultaneous determination of vitamins D3, E and K1 and retinyl palmitate in cattle plasma by liquid chromatography with a narrow-bore column, *J.Chromatogr.B*, **1994**, *660*, 49–55.

SAMPLE
Matrix: formula
Sample preparation: Weigh out formula containing 12 IU vitamin D3, add 15 mL EtOH:isopropanol 95:5, add 400 mg ascorbic acid, shake vigorously for 5 s, add 7.5 g solid KOH, shake vigorously for 20 s, vent pressure, heat at 75° for 30 min, cool, rinse tube into separatory funnel with 5 mL EtOH:isopropanol 95:5, add 130 mL ethyl ether, shake vigorously for 30 s, add 130 mL petroleum ether, shake vigorously for 30 s, discard all but 1 mL of aqueous layer, add 50 mL water, shake vigorously for 15 s, repeat wash, add 15 mL EtOH:isopropanol 95:5, repeat

wash. Evaporate the organic layer to dryness under reduced pressure at <50°, reconstitute with 50 mL acetone, evaporate to dryness, transfer to small tube with three 10 mL portions of ether, evaporate to dryness, reconstitute with 1 mL hexane, vortex for 5 s, inject 320-370 μL on to a 30 × 4.6 Spheri-5 amino + 250 × 4.6 Partisil-5 PAC silica column and elute with hexane:amyl alcohol 99.2:0.8 at 2 mL/min, monitor at UV 254 and collect vitamin D3 fraction (elute peaks after vitamin D3 at 4 mL/min), evaporate collected fraction to dryness under a stream of nitrogen at 50°, reconstitute with 1 mL hexane, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 3 μm Apex I silica (Jones Chromatography)
Mobile phase: Hexane:amyl alcohol 99.85:0.15
Flow rate: 3
Injection volume: 250
Detector: UV 254

CHROMATOGRAM
Retention time: 14-18

KEY WORDS
protect from light; normal phase

REFERENCE
Tanner,J.T.; Barnett,S.A.; Mountford,M.K. Analysis of milk-based infant formula. Phase IV. Iodide, linoleic acid, and vitamins D and K: U.S. Food and Drug Administration-Infant Formula Council: Collaborative study, *J.AOAC Int.*, **1993**, *76*, 1042–1056.

SAMPLE
Matrix: formula
Sample preparation: Condition a 2.8 mL 500 mg silica SPE cartridge with 4 mL dichloromethane:isopropanol 80:20 and 5 mL dichloromethane:isopropanol 99.8:0.2. 15 mL Formula + 4 mL 46 ng/mL vitamin D_2 in EtOH + 15 mL ethanolic KOH, shake at 60° for 30 min, cool to room temperature, add 15 mL water, add 60 mL hexane, shake vigorously for 1.5 min, let stand for 10 min, discard aqueous layer. Wash the hexane layer with 15 mL water, add 15 mL water and 1 drop phenolphthalein to the hexane layer, add 10% acetic acid dropwise with shaking until the aqueous layer is colorless. Filter the hexane layer through anhydrous sodium sulfate, wash through with a few mL hexane, evaporate to dryness under reduced pressure at 40°, reconstitute with 2 mL dichloromethane:isopropanol 99.8:0.2, add to the SPE cartridge, rinse flask with 1 mL dichloromethane:isopropanol 99.8:0.2, add rinse to cartridge, wash with 2 mL dichloromethane:isopropanol 99.8:0.2, elute with 7 mL dichloromethane:isopropanol 99.8:0.2. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 1 mL MeCN, inject an aliquot. (Prepare ethanolic KOH by dissolving 140 g KOH in 310 mL EtOH, add 50 mL water. Prepare fresh each day.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm C18 (not end-capped)
Mobile phase: Gradient. MeCN:MeOH:ethyl acetate 91:9:0 for 28 min, to 0:0:100 over 0.5 min, maintain at 0:0:100 for 2.5 min, return to initial conditions over 0.5 min, re-equilibrate for 2.5 min.
Column temperature: 27
Flow rate: 0.7 for 28 min, to 2.5 over 0.5 min, maintain at 2.5 for 4.5 min, return to initial conditions over 1 min
Injection volume: 250
Detector: UV 265

CHROMATOGRAM
Retention time: 23
Internal standard: vitamin D_2 (19.5)

KEY WORDS
protect from light and oxygen; SPE

REFERENCE

Sliva,M.G.; Sanders,J.K. Vitamin D in infant formula and enteral products by liquid chromatography: Collaborative study, *J.AOAC Int.*, **1996**, *79*, 73–80.

SAMPLE

Matrix: formulations

Sample preparation: Powder tablets, weigh out powder equivalent to 200 IU vitamin D3, add 5 μg vitamin D2 and 10 mL EtOH:water 50:50. Extract with 15 mL hexane 3 times. Remove the organic layer and dry it under reduced pressure. Dissolve the residue in 1 mL MeOH:water 90:10 and inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4 5 μm TSK-gel ODS 80TM (TOSOH, Japan)
Mobile phase: MeOH:water 90:10
Flow rate: 1
Injection volume: 20
Detector: MS, Hitachi M-1000, APCI interface, drift voltage 20 V, focus voltage 120 V, vaporizer 399°, desolvation chamber 200°, multiplier voltage 2 kV

CHROMATOGRAM

Retention time: 14.8
Internal standard: vitamin D2 (14.4)
Limit of detection: 400 pg

KEY WORDS

tablets

REFERENCE

Adachi,T.; Nishio,M.; Yunoki,N.; Hayashi,H. Determination of vitamin D_3 and D_2 in multi-vitamin tablets by high-performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometry, *Anal.Sci.*, **1994**, *10*, 457–460.

SAMPLE

Matrix: milk

Sample preparation: Mix 50 mL milk with 30 mL ethanolic KOH (10:30). Saponify the mixture at 80° for 20min. Extract twice with 10 mL n-hexane. Evaporate the extracts to dryness, reconstitute the residue with 1 mL mobile phase, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5μm C18 (Alltech)
Mobile phase: MeOH:EtOH 80:20 (A) or EtOH:water 95:5 (B)
Flow rate: 1
Injection volume: 5
Detector: UV 250

CHROMATOGRAM

Retention time: 7.3 (A), 10 (B)

OTHER SUBSTANCES

Extracted: isotretinoin, retinal, tretinoin, vitamin A, vitamin D2, vitamin E, vitamin K1, vitamin K2

REFERENCE

Gong,B.Y.; Ho,J.W. Simultaneous separation and detection of ten common fat-soluble vitamins in milk, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2389–2397.

SAMPLE

Matrix: milk

Sample preparation: Dilute milk to 30% with water, mix with reagent and pass through a 5 m × 0.5 mm i.d. PTFE tube knotted reactor at 1.25 mL/min, mix with 2.5 M acetic acid, pass

onto column a Sep-Pak Plus C18 SPE cartridge for 5 min, wash column A with MeOH:water 40:60 for 4 min, elute column A with MeOH for 4 min, inject the last 100 μL aliquot of the eluate. (Reagent was 50 mL EtOH + 15 mL 60% aqueous NaOH + 5 mL 10% ascorbic acid.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm RP18 (Brownlee)
Column: 220 × 4.6 5 μm OD-224 RP18 (Brownlee)
Mobile phase: MeOH:water 99:1 containing 2.5 mM acetic acid-sodium acetate
Flow rate: 1
Injection volume: 100
Detector: E, glassy carbon working electrode +1300 mV

CHROMATOGRAM
Retention time: 10
Limit of detection: 1.77 μM

OTHER SUBSTANCES
Extracted: vitamin A, vitamin E

KEY WORDS
SPE

REFERENCE
Delgado-Zamarreño,M.M.; Sanchez-Perez,A.; Gomez-Perez,M.C.; Hernandez-Mendez,J. Directly coupled sample treatment-high-performance liquid chromatography for on-line automatic determination of liposoluble vitamins in milk, *J.Chromatogr.A*, **1995**, *694*, 399–406.

SAMPLE
Matrix: solutions
Sample preparation: Mix 100 μL 10 μg/mL 7-dehydrocholesterol in ethyl acetate with 100 μL ethyl acetate containing 20-50 equivalents 4-phenyl-1,2,4-triazoline-3,5-dione, after 30 min, inject an aliquot.

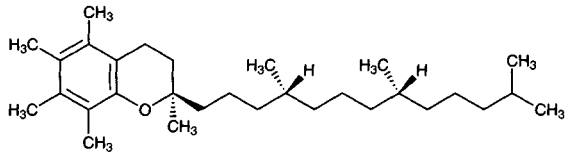
HPLC VARIABLES
Column: 150 × 4.6 5 μm YMC-Gel C8-120-S5
Mobile phase: MeCN:water 90:10
Flow rate: 1
Detector: UV 265

CHROMATOGRAM
Retention time: 7

KEY WORDS
derivatization

REFERENCE
Shimada,K.; Oe,T.; Mizuguchi,T. Cookson-type reagents: highly sensitive derivatization reagents for conjugated dienes in high-performance liquid chromatography, *Analyst*, **1991**, *116*, 1393–1397.

Vitamin E



Molecular formula: $C_{29}H_{50}O_2$
Molecular weight: 430.71
CAS Registry No.: 59-02-9, 58-95-7 (acetate d-form), 52225-20-4 (acetate dl-form), 43119-47-7 (nicotinate)
Merck Index: 10159

SAMPLE

Matrix: blood
Sample preparation: Dilute 100 μL serum with 900 μL 12.62 mg/mL pyrogallol in EtOH, filter (450 μm cellulose disk), cool at 15° in the autosampler, inject a 300 μL aliquot onto column A and elute to waste with mobile phase A. After 3 min backflush the contents of column A onto column B with mobile phase B, after another 1 min remove column A from the circuit. Elute column B with mobile phase B for another 7.5 min then elute with mobile phase C. Monitor the effluent from column B.

HPLC VARIABLES

Column: A 10 × 4.6 13 μm TSK BSA-80Ts; B 15 × 3.2 5 μm TSK ODS-80Ts + 150 × 4.6 5 μm TSKgel ODS-80Ts
Mobile phase: A 200 mM sodium dodecyl sulfate solution:EtOH 70:30 containing 200 mM ethylenediaminetetraacetic acid 4 sodium salt and 0.3% phosphoric acid; B EtOH:water 80:20; C EtOH:water 87:13
Column temperature: 40
Flow rate: A 1.5; B 1; C 1
Injection volume: 300
Detector: F ex 298 em 325

CHROMATOGRAM

Retention time: 14
Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Extracted: vitamin A (ex 340 em 460)

KEY WORDS

serum; column-switching

REFERENCE

Moriyama,H.; Yamasaki,H.; Masumoto,S.; Adachi,K.; Katsura,N.; Onimaru,T. Rapid determination of vitamins A and E in serum with surfactant as a diluent by column-switching high-performance liquid chromatography, *J.Chromatogr.A*, **1998**, *798*, 125–130.

SAMPLE

Matrix: blood
Sample preparation: Suspend erythrocytes in pH 7.4 isotonic phosphate buffered saline (140 mM NaCl containing 10 mM phosphate buffer), centrifuge at 2900 g for 15 min at 4°. Homogenize erythrocyte precipitate with the same volume water, centrifuge at 725 g and 16260 g for 15 min to give cytosol component and membrane respectively. Filter samples (Millex HV13 0.45 μm) before injection.

HPLC VARIABLES

Column: 150 × 4.6 10 μm Pecosphere HS-5 HC ODS (Perkin-Elmer, USA)
Mobile phase: MeOH:water 93.5:6.5 containing 2.8 mm 1-octanesulfonic acid sodium salt
Flow rate: 1.6
Injection volume: 20

Detector: UV 282

OTHER SUBSTANCES
Also analyzed: tocopherol succinate, tocopherol succinate-3-glucose

KEY WORDS
erythrocytes; cow

REFERENCE
Bonina,F.; Lanza,M.; Montenegro,L.; Salerno,L.; Smeriglio,P.; Trombetta,D.; Saija,A. Transport of α-tocopherol and its derivatives through erythrocyte membranes, *Pharm.Res.*, **1996**, *13*, 1343–1347.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Serum + 2 mL EtOH + 10 mL hexane, mix for 30 s, centrifuge at 3000 rpm for 5 min, store the hexane layer at 15°, repeat the extraction with 10 mL hexane. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute with 200 μL isopropanol, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm TSKgel ODS-80Ts
Mobile phase: Gradient. EtOH:water 80:20 for 11.5 min then 87:13 (step gradient)
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: F ex 298 em 325

CHROMATOGRAM
Retention time: 7.5

OTHER SUBSTANCES
Extracted: vitamin A (F ex 340 em 460)

KEY WORDS
serum

REFERENCE
Moriyama,H.; Yamasaki,H.; Masumoto,S.; Adachi,K.; Katsura,N.; Onimaru,T. Rapid determination of vitamins A and E in serum with surfactant as a diluent by column-switching high-performance liquid chromatography, *J.Chromatogr.A*, **1998**, *798*, 125–130.

SAMPLE
Matrix: blood
Sample preparation: Add 100 μL 837 ng/mL retinyl acetate in EtOH and 400 μL EtOH to 500 μL plasma, vortex for 30 s, add 2 mL hexane, vortex for 30 s, centrifuge at 1500 rpm for 5 min. Remove the upper hexane layer, add 2 mL hexane to the remaining lower layer, reextract. Evaporate the combined hexane layers to dryness under nitrogen at 40°, reconstitute the residue in 200 μL 30.28 μg/mL (α-tocopheryl acetate in EtOH, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm 100 Å pore size Supelguard column (Supelco)
Column: 250 × 4.6 5 μm 100 Å pore size Suplex pKb-100 RP (Supelco)
Mobile phase: MeOH:MTBE:water 80:20:5
Column temperature: 0
Flow rate: 0.8
Injection volume: 50
Detector: UV 292

CHROMATOGRAM
Retention time: 19.9 (α-tocopherol)

Internal standard: α-tocopheryl acetate (16.6)

OTHER SUBSTANCES
Extracted: γ-tocopherol, retinyl acetate

KEY WORDS
plasma

REFERENCE
Lane,J.R.; Webb,L.W.; Acuff,R.V. Concurrent liquid chromatographic separation and photodiode array detection of retinol, tocopherols, all-trans-α-carotene, all-trans-β-carotene and the mono-cis isomers of β-carotene in extracts of human plasma, *J.Chromatogr.A*, **1997**, *787*, 111–118.

SAMPLE
Matrix: blood
Sample preparation: Centrifuge 200 μL serum, add 200 μL IS and 200 μL EtOH, vortex on orbital shaker for 5 min, add 200 μL water and 500 μL hexane, vortex for 10 min, centrifuge at 2000 g for 10 min at 17°, remove 300 μL upper organic layer. Re-extract with 300 μL hexane, vortex for 10 min, centrifuge at 4000 g for 10 min at 17°, remove 300 μL upper organic layer. Combine organic layers, and evaporate to dryness under vacuum in 15 min. Reconstitute the residue with 300 μL MeOH:EtOH:hexane 88:10:2, vortex for 10 min, inject a 40 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 3 μm Adsorbosphere HS C18 + 150 × 4.6 3 μm Adsorbosphere HS C18 in series
Mobile phase: Step gradient. A was MeCN:MeOH 60:40 containing 0.05% acetic acid. B was MeCN:MeCN:dichloromethane 45.6:30.4:24 containing 0.04% acetic acid. A:B 100:0 for 7 min, to 0:100 7.1 min after injection to 17.4 min, re-equilibrate with A for 5.6 min
Column temperature: 37
Flow rate: 0.9
Injection volume: 40
Detector: UV 292

CHROMATOGRAM
Retention time: 12.4
Internal standard: tocol (UV 292) (10.1), echinenone (UV 450) (12.8)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: canthaxanthine (UV 473), α-carotene (UV 450), β-carotene (UV 450), β-cryptoxanthine (UV 450), lutein (UV 450), lycopene (UV 473), vitamin A (UV 325), zeaxanthin (UV 450), nonidentified carotenoids

KEY WORDS
serum; protect from light

REFERENCE
Steghens,J.-P.; van Kappel,A.L.; Riboli,E.; Collombel,C. Simultaneous measurement of seven carotenoids, retinol and α-tocopherol in serum by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *694*, 71–81.

SAMPLE
Matrix: blood
Sample preparation: Add 1 mL 0.5 μg/mL retinyl acetate, 1 μg/mL retinyl palmitate, and 25 μg/mL α-tocopheryl acetate in EtOH to 1 mL serum or plasma while continuously vortexing, add 3 mL hexane, vortex for 2 min, centrifuge at 2500 g for 2 min, remove the upper phase, add 2 mL hexane to the lower layer, repeat extraction. Combine the upper layers and evaporate them to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL mobile phase, inject a 40 μL aliquot,

HPLC VARIABLES
Guard column: C18 (Waters)

Column: 5 μm Biophase ODS C18 (Bioanalytical Systems)
Mobile phase: MeCN:chloroform:isopropanol:water 78:16:3.5:2.5
Flow rate: 2
Injection volume: 40
Detector: UV 292 (UV 460 for carotenoids)

CHROMATOGRAM
Retention time: 6.74
Internal standard: retinyl acetate (3.07), retinyl palmitate (18.66), α-tocopheryl acetate (8.33)

OTHER SUBSTANCES
Extracted: β-carotene, vitamin A (retinol), gamma-tocopherol, α-carotene, lycopene, cryptoxanthin

KEY WORDS
serum; plasma

REFERENCE
Kaplan,L.A.; Miller,J.A.; Stein,E.A.; Stampfer,M.J. Simultaneous, high-performance liquid chromatographic analysis of retinol, tocopherols, lycopene, and α- and β-carotene in serum and plasma, *Methods Enzymol.*, **1990**, *189*, 155–167.

SAMPLE
Matrix: blood
Sample preparation: 250 μL Serum + 25 μL 80 μg/mL tocol in EtOH + 250 μL 20 μg/mL BHT (butylated hydroxytoluene) in EtOH + 1.5 mL hexane, vortex for 1 min, remove 1 mL of upper layer, add 500 μL hexane, vortex for 1 min, remove 300 μL of upper layer. Combine the hexane extracts, evaporate to dryness under a stream of inert gas. Reconstitute in 250 μL 20 μg/mL BHT in EtOH, sonicate, centrifuge if necessary, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Vydac 201TP54 (wide pore, polymerically bonded C18)
Mobile phase: Gradient. A was MeOH:n-butanol:water 75:10:15 containing 50 mM ammonium acetate, pH 5.5. B was MeOH:n-butanol:water 88:10:2 containing 50 mM ammonium acetate, pH 5.5. A:B 100:0 for 3 min, to 0:100 over 15 min, maintain at 0:100 for 17 min
Injection volume: 25
Detector: UV 325 for 7 min, UV 295 for 13 min, UV 450 for 14 min or E, glassy carbon electrode, Ag/AgCl reference electrode +1050 mV for retinol, +900 mV for tocol, +750 mV for α-tocopherol, +700 mV for β-carotene

CHROMATOGRAM
Retention time: 18.5
Internal standard: tocol (13)
Limit of detection: 650 ng/mL (E), 96 μg/mL (UV)

OTHER SUBSTANCES
Extracted: β-carotene, vitamin A (retinol), gamma-tocopherol, lutein, zeaxanthin, cryptoxanthin, α-carotene, 9-cis-β-carotene

KEY WORDS
serum

REFERENCE
MacCrehan,W.A. Determination of retinol, α-tocopherol, and β-carotene in serum by liquid chromatography, *Methods Enzymol.*, **1990**, *189*, 172–181.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 100 μL EtOH + 100 μL α-tocopheryl acetate in EtOH, vortex for 5 s, add 500 μL hexane, vortex for 2 min, centrifuge at 700 g for 5 min. Remove 250

μL of the hexane layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, mix for 2 min, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 10 μm Spheri-10 RP18
Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:dichloromethane:MeOH 70:20:10
Flow rate: 1.2
Injection volume: 50
Detector: UV 325 for 3.5 min, UV 291 for 4.5 min, UV 450 for 6 min

CHROMATOGRAM
Retention time: 5.31
Internal standard: α-tocopheryl acetate (6.30)
Limit of detection: 180 nM

OTHER SUBSTANCES
Extracted: beta carotene, vitamin A

KEY WORDS
protect from light; serum

REFERENCE
Arnaud,J.; Fortis,I.; Blachier,S.; Kia,D.; Favier,A. Simultaneous determination of retinol, α-tocopherol and β-carotene in serum by isocratic high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *572*, 103–116.

SAMPLE
Matrix: blood
Sample preparation: 2.5 mL Plasma + 2.5 mL 18 ng/mL IS1 and 10 ng/mL IS2 in EtOH, shake vigorously for 20 s, centrifuge at 1200 g for 5 min, add 5 mL diethyl ether, shake vigorously, centrifuge for 5 min, extract twice more with 5 mL ether. Combine ether layers, wash with 15 mL 5% NaCl, dry over sodium sulfate, evaporate to dryness under vacuum at 35°. Dissolve residue in 1-2 mL dichloromethane, filter (0.45 μm). Evaporate to dryness under a stream of nitrogen, make up to 100 μL with MeCN:MeOH:dichloromethane:hexane 45:10:22.5:22.5, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 5 μm Spheri-5-C18 (Brownlee)
Column: 250 × 4.6 5 μm Microsorb C18 (Rainin)
Mobile phase: Gradient. MeCN:MeOH:dichloromethane:hexane 85:10:2.5:2.5 for 10 min then to 45:10:22.5:22.5 over 30 min, re-equilibrate for 15 min
Flow rate: 0.7
Injection volume: 20
Detector: UV 290

CHROMATOGRAM
Retention time: 24
Internal standard: IS1 ethyl β-apo-8'-carotenate (18), IS2 (3R)-8'-apo-β-carotene-3,8'-diol (5)

OTHER SUBSTANCES
Extracted: carotenoids, β-carotene, vitamin A (retinol)

KEY WORDS
plasma; handle under yellow lights

REFERENCE
Khachik,F.; Beecher,G.R.; Goli,M.B.; Lusby,W.R.; Smith,J.C.,Jr. Separation and identification of carotenoids and their oxidation products in the extracts of human plasma, *Anal.Chem.*, **1992**, *64*, 2111–2122.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum or plasma + 200 μL 25 μg/mL tocopheryl acetate in EtOH, vortex, add 400 μL butanol:ethyl acetate 50:50, mix for 1 min, add 20 mg sodium sulfate, vortex for 1 min, let stand at -20° for 20 min, centrifuge at 15000 g for 2 min, inject a 10 μL aliquot of the upper organic layer.

HPLC VARIABLES
Guard column: 5 μm C18
Column: 110 × 4.7 5 μm Partisphere 5 C18 (Whatman)
Mobile phase: MeOH:butanol:water 89.5:5:5.5
Column temperature: 45
Flow rate: 1.5
Injection volume: 10
Detector: UV 340 for 3 min, UV 290 for 1.5 min, UV 280 for 10.5 min, UV 450 for 7 min

CHROMATOGRAM
Retention time: 4.0
Internal standard: tocopheryl acetate (5.3)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: α-carotene, β-carotene, lycopene, δ-tocopherol, gamma-tocopherol, vitamin A, xanthophyll

KEY WORDS
serum; plasma; protect from light

REFERENCE
Lee,B.L.; Chua,S.C.; Ong,H.Y.; Ong,C.N. High-performance liquid chromatographic method for routine determination of vitamins A and E and β-carotene in plasma, *J.Chromatogr.*, **1992**, *581*, 41–47.

SAMPLE
Matrix: blood
Sample preparation: Dilute 1 mL serum 0.5-5 times with saline. Add 1 mL 10 μg/mL IS in EtOH to 1 mL diluted serum dropwise while vortexing, add 1.5 mL n-heptane, vortex for 1 min, centrifuge at 3000 rpm (Labofuge) for 15 min. Remove 1.3 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 40 μL MeCN:THF 50:50, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 200 × 2.1 5 μm ODS Hypersil
Mobile phase: MeCN:water:THF 81.3:5.7:13
Column temperature: 40
Flow rate: 0.4
Injection volume: 5
Detector: UV 292

CHROMATOGRAM
Retention time: 5.585
Internal standard: α-tocopherol acetate (6.185)
Limit of detection: 15 ng

OTHER SUBSTANCES
Extracted: probucol, gamma-tocopherol, vitamin A (retinol), lycopene, α-carotene, β-carotene, metabolites

KEY WORDS
serum

REFERENCE
Schäfer Elinder,L.; Walldius,G. Simultaneous measurement of serum probucol and lipid-soluble antioxidants, *J.Lipid Res.*, **1992**, *33*, 131–137.

SAMPLE
Matrix: blood
Sample preparation: 20-500 μL Serum + 2 volumes EtOH + 1 mL ethyl acetate + 4-7 μL of a solution containing 16 mg/mL tocopheryl acetate, 2-3 μg/mL canthaxanthin, and 10 μg/mL retinoic acid, vortex for 30 s, centrifuge for 30 s, extract the pellet twice with 0.5-1 mL portions of ethyl acetate, extract the pellet with 0.5-1 mL hexane. Combine the supernatants, add 500 μL water, vortex, centrifuge. Remove the upper organic layer and evaporate it to dryness under a stream of argon, reconstitute the residue in 100 μL MeOH:dichloromethane 2:1, inject a 10-90 μL aliquot.

HPLC VARIABLES
Guard column: C18 (Upchurch)
Column: 300 × 3.9 5 μm Resolve C18 (Waters)
Mobile phase: MeCN:dichloromethane:MeOH:1-octanol 90:15:10:0.1
Flow rate: 1
Injection volume: 10-90
Detector: UV 290

CHROMATOGRAM
Retention time: 10
Internal standard: tocopheryl acetate, canthaxanthin, retinoic acid

OTHER SUBSTANCES
Extracted: beta carotene (UV 450), carotenoids (UV 450), vitamin A (UV 325)

KEY WORDS
protect from light; serum

REFERENCE
Barua,A.B.; Kostic,D.; Olson,J.A. New simplified procedures for the extraction and simultaneous high-performance liquid chromatographic analysis of retinol, tocopherols and carotenoids in human serum, *J.Chromatogr.*, **1993**, *617*, 257–264.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum or plasma + 500 μL EtOH containing 4.27 μM retinyl acetate and 0.31 μM echinenone, rotamix for 30 s, add 2 mL n-hexane, rotamix for 30 s, centrifuge at 2000 g for 2 min, repeat extraction with 2 mL n-hexane. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 50 μL THF, make up to 200 μL with EtOH, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 4.6 5 μm Spherisorb ODS1
Column: 250 × 4.6 5 μm Spherisorb ODS1
Mobile phase: Gradient. A was MeCN:MeOH 20:80 containing 100 mM ammonium acetate. B was 100 mM ammonium acetate in water. A:B from 90:10 to 100:0 over 12 min, maintain at 100:0 for 10 min, re-equilibrate at initial conditions for 5 min
Flow rate: 2
Injection volume: 50
Detector: UV 325 for 7.5 min, UV 292 for 5.5 min, then UV 450

CHROMATOGRAM
Retention time: 11.87
Internal standard: retinyl acetate (5.96), echinenone (15.15)
Limit of detection: 5.80 μM

OTHER SUBSTANCES
Extracted: β-carotene, cryptoxanthin, lutein, lycopene, vitamin A

KEY WORDS
plasma; protect from light; serum

REFERENCE
Zaman,Z.; Fielden,P.; Frost,P.G. Simultaneous determination of vitamins A and E and carotenoids in plasma by reversed-phase HPLC in elderly and younger subjects, *Clin.Chem.*, **1993**, *39*, 2229–2234.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma or serum + 200 μL 850 ng/mL retinyl acetate in EtOH, mix for 1 min, add 1 mL 0.4 g/L BHT (2,6-di-tert-butyl-4-methylphenol) in n-hexane, shake on a mechanical shaker for 10 min, centrifuge at 2000 g for 5 min, remove 800 μL of the supernatant, evaporate to dryness at 40° under a stream of nitrogen, reconstitute in 100 μL MeCN:THF:MeOH 68:22:7, inject a 15 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Lichrosorb RP18
Column: 250 × 4.6 5 μm Nucleosil 100-5 C18
Mobile phase: MeCN:THF:MeOH 68:22:7 made up to 100 with 1% ammonium acetate
Flow rate: 1.5
Injection volume: 15
Detector: UV 325 for 3 min, UV 450 for 1.9 min, UV 290 for 2.5 min, UV 470 for 4.6 min, UV 450 for 3 min, then UV 325 for rest of run

CHROMATOGRAM
Retention time: 6.3
Internal standard: retinyl acetate (2.7)
Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Extracted: β-carotene, vitamin A (retinol), lutein, lycopene, α-carotene, zeaxanthin, trans β-carotene, δ-tocopherol

KEY WORDS
plasma; serum; protect from sunlight

REFERENCE
Bui,M.H. Simple determination of retinol, α-tocopherol and carotenoids (lutein, all-*trans*-lycopene, α- and β-carotenes) in human plasma by isocratic liquid chromatography, *J.Chromatogr.B*, **1994**, *654*, 129–133.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 2 mL EtOH, vortex for 3 min, add 3 mL hexane, vortex for 5 min, centrifuge at 1500 g for 10 min, repeat extraction with 3 mL hexane. Combine the hexane layers and wash with 2 mL MeOH:water 9:1. Remove the upper organic layer and filter (0.45 μm) it, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 33.4 μL EtOH, inject a 5 μL aliquot.

HPLC VARIABLES
Guard column: ODS
Column: 125 × 4 5 μm LiChrospher 100 RP-18 or 100 × 2.1 3 μm Spherisorb ODS-2
Mobile phase: Gradient. A was MeOH:water 99:1. B was MeOH:THF 70:30. A:B 100:0 for 2 min, 5:95 for 3.5 min, 0:100 for 4.5 min, re-equilibrate for 2 min (step gradients).
Flow rate: 1.5 (125 × 4 column) or 0.2 (100 × 2.1 column)
Injection volume: 5
Detector: UV 284

CHROMATOGRAM
Retention time: 5 (125 × 4 column), 6 (100 × 2.1 column)
Limit of detection: 2.8 ng (100 × 2.1 column), 106.3 ng (125 × 4 column)

OTHER SUBSTANCES
Extracted: retinyl palmitate (UV 328), vitamin D3 (cholecalciferol) (UV 265), vitamin K1 (UV 250)

KEY WORDS
cow; plasma; protect from light; degas stock solutions with helium; narrow bore

REFERENCE
Gomis,D.B.; Escotet Arias,V.J.; Fidalgo Alvarez,L.E.; Gutiérrez Alvarez,M.D. Simultaneous determination of vitamins D3, E and K1 and retinyl palmitate in cattle plasma by liquid chromatography with a narrow-bore column, *J.Chromatogr.B*, **1994**, *660*, 49–55.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 200 μL nonapreno-β-carotene and retinyl butyrate in EtOH, vortex for 10 s, add 1 mL hexane, vortex for 30 s, centrifuge at 1500 g for 5 min. Remove 900 μL of the hexane layer and evaporate it to a waxy or glassy consistency (not dryness) under vacuum, dissolve in 100 μL EtOH, add 100 μL MeCN, vortex, filter (0.45 μm), inject a 30 μL aliquot.

HPLC VARIABLES
Column: 1540 × 4.6 5 μm Ultramex C18 (Phenomenex)
Mobile phase: MeCN:EtOH 50:50 containing 0.1 mL/L diethylamine
Column temperature: 29
Flow rate: 0.9
Injection volume: 30
Detector: UV 300

CHROMATOGRAM
Retention time: 4.60
Internal standard: nonapreno-β-carotene (9.5, UV 450), retinyl butyrate (3.5, UV 300)
Limit of detection: 460 nM

OTHER SUBSTANCES
Extracted: β-carotene, vitamin A (retinol), lutein, zeaxanthin, β-cryptoxanthin, lycopene, α-carotene, retinyl linoleate, retinyl oleate, retinyl palmitate, retinyl stearate

KEY WORDS
serum; use gold fluorescent lamps; hold sample at 4° before injection

REFERENCE
Sowell,A.L.; Huff,D.L.; Yeager,P.R.; Caudill,S.P.; Gunter,E.W. Retinol, α-tocopherol, lutein/zeaxanthin, β-cryptoxanthin, lycopene, α-carotene, trans-β-carotene, and four retinyl esters in serum determined simultaneously by reversed-phase HPLC with multiwavelength detection, *Clin.Chem.*, **1994**, *40*, 411–416.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 2.5 mL EtOH, mix for 5 min, add 5 mL n-hexane, mix vigorously, centrifuge at 2000 g for 5 min, repeat extraction with 3 mL n-hexane. Combine the n-hexane layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in dichloromethane, inject an aliquot.

HPLC VARIABLES
Guard column: μBondapak C18 Guard-Pak + 50 mm long C18 ODS(4) (Shimadzu)
Column: 250 × 4.6 5 μm Vydac 201 TP 54 C18
Mobile phase: MeOH:MeCN 90:10 (Every 100 injections wash column with MeOH:MeCN:dichloromethane 8:1:1.)
Flow rate: 1
Detector: UV 291

CHROMATOGRAM
Retention time: 6.9

OTHER SUBSTANCES
Extracted: beta carotene (UV 451), vitamin A (UV 324)

KEY WORDS
serum

REFERENCE
Ben-Amotz,A. Simultaneous profiling and identification of carotenoids, retinols, and tocopherols by high performance liquid chromatography equipped with three-dimensional photodiode array detection, *J.Liq.Chromatogr.*, **1995**, *18*, 2813–2825.

SAMPLE
Matrix: blood
Sample preparation: 400 μL Plasma + 2 mL MeOH, vortex for 5 min, centrifuge at 1200 g for 10 min, filter (0.45 μm), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 200 × 4.6 10 μm LiChrosorb RP-18
Mobile phase: MeOH
Flow rate: 1
Injection volume: 20
Detector: UV 292

CHROMATOGRAM
Retention time: 11.1 (vitamin E), 14.5 (vitamin E acetate)
Limit of detection: 750 ng/mL (vitamin E), 200 ng/mL (vitamin E acetate)

KEY WORDS
plasma; dog; pharmacokinetics

REFERENCE
Santiago Torrado,D.; Jimenez Caballero,E.; Cadorniga,R.; Torrado,J. A selective liquid chromatography assay for the determination of dl-α-tocopherol acetate on plasma samples, *J.Liq.Chromatogr.*, **1995**, *18*, 1251–1264.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 200 μL 650 ng/mL tocopheryl acetate in MeOH, vortex for 30 s, add 200 μL n-hexane, shake for 15 min, centrifuge at 3000 rpm for 10 min. Remove 120 μL of the hexane layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 20 μL dichloromethane, add 100 μL MeCN:MeOH 50:50, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4 5 μm Nucleosil C18
Mobile phase: MeCN:MeOH:dichloromethane 50:45:5
Flow rate: 0.7
Injection volume: 50
Detector: F ex 325 em 480 for 5 min, then ex 295 em 330 for 10 min, then ex 325 em 480

CHROMATOGRAM
Retention time: 7.3
Internal standard: tocopheryl acetate (9.3)

OTHER SUBSTANCES
Extracted: vitamin A, γ-tocopherol, retinyl palmitate, β-carotene (UV 450), α-carotene (UV 450)

KEY WORDS
serum

REFERENCE

Yakushina,L.; Taranova,A. Rapid HPLC simultaneous determination of fat-soluble vitamins, including carotenoids, in human serum, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 715–718.

SAMPLE

Matrix: blood

Sample preparation: Mix 500 μL plasma with 30 μL 500 mM sodium bisulfite, deoxygenate with nitrogen, add 500 μL EtOH (deoxygenated with nitrogen), mix vigorously for 3 min, add 2.5 mL n-hexane (deoxygenated with nitrogen), mix, centrifuge at 4° at 3000 rpm for 5 min. Remove the hexane layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase (deoxygenated with nitrogen), inject an aliquot

HPLC VARIABLES

Column: 80 × 4.6 3 μm MC MEDICAL C18 (MC Medical, Tokyo)

Mobile phase: MeOH:water 96:4 containing 40 mM sodium perchlorate (deoxygenate with nitrogen)

Column temperature: 35

Flow rate: 1

Injection volume: 100

Detector: E, ESA, Model 5100A, Model 5020 guard cell before injector -0.45 V, Model 5021 conditioning cell after column but before analytical cell -0.45 V, Model 5011 analytical cell at -0.45 V and +0.40 V (monitored)

CHROMATOGRAM

Retention time: 7.2

Limit of detection: 50 pg

OTHER SUBSTANCES

Extracted: β-tocopherol, gamma-tocopherol, δ-tocopherol, α-tocopherolquinone

KEY WORDS

recirculate mobile phase; rat; plasma

REFERENCE

Takeda,H.; Shibuya,T.; Yanagawa,K.; Kanoh,H.; Takasaki,M. Simultaneous determination of α-tocopherol and α-tocopherolquinone by high-performance liquid chromatography and coulometric detection in the redox mode, *J.Chromatogr.A*, **1996**, *722*, 287–294.

SAMPLE

Matrix: blood, tissue

Sample preparation: Plasma. 100 μL Plasma + 100 μL 0.9% NaCl + 200 μL MeOH, vortex for 30 s, let stand for 10 min, add 400 μL chloroform, vortex for 4 min. Remove the chloroform layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in MeOH, inject an aliquot. Tissue. Homogenize (liver with Mikro-dismembrator II in liquid nitrogen; other tissue with Ultra-Turrax) tissue with 3 mL 1% acetic acid containing 1 mg/mL ascorbic acid and 10 mM EDTA, add 2 mL MeOH, vortex for 30 s, let stand for 10 min, add 4 mL chloroform, vortex for 4 min. Remove the chloroform layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in MeOH, inject an aliquot.

HPLC VARIABLES

Column: 125 × 4.6 3 μm Hypersil ODS

Mobile phase: MeCN:dichloromethane:MeOH:water 70:10:15:5

Flow rate: 0.5 for 13 min, to 1 over 1 min, maintain at 1 for 10 min, to 1.5 over 1 min, maintain at 1.5 for 21 min, to 2 over 1 min, maintain at 2 for 10 min, return to 0.5 over 1 min, maintain at 0.5 for 2 min.

Injection volume: 50

Detector: UV 292

CHROMATOGRAM

Retention time: 17

Limit of detection: 60 ng/mL

OTHER SUBSTANCES
Extracted: beta carotene (UV 445), vitamin A (UV 350)

KEY WORDS
rat; protect from light; liver; plasma; lung

REFERENCE
Van Vliet,T.; Van Schaik,F.; Van Schoonhoven,J.; Schrijver,J. Determination of several retinoids, carotenoids and E vitamers by high-performance liquid chromatography. Application to plasma and tissues of rats fed a diet rich in either β-carotene or canthaxanthin, *J.Chromatogr.*, **1991**, *553*, 179–186.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a Lichrolut RP-18 (Merck) SPE cartridge with 3 mL MeOH and 3 mL water. Mix 40 μL plasma or 100 μL urine with twice the volume of MeCN for 2 min, add 100 μL water, centrifuge at 3500 rpm for 15 min, evaporate the supernatant under nitrogen at 45° to remove the organic solvents, add slowly to the SPE cartridge, discard the effluent, elute with 3 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 45°. Reconstitute the residue with 500 μL MeOH containing 3.5 μg/mL IS. Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 220 × 4.6 5 μm Spherisorb RP-18
Mobile phase: MeCN:MeOH 70:30
Flow rate: 1.5
Injection volume: 10
Detector: UV 290

CHROMATOGRAM
Retention time: 7.78 (vitamin E), 8.79 (vitamin E acetate)
Internal standard: anthraquinone (1.89)
Limit of detection: 5 ng

OTHER SUBSTANCES
Extracted: vitamin A

KEY WORDS
plasma; SPE

REFERENCE
Papadoyannis,I.N.; Tsioni,G.K.; Samanidou,V.F. Simultaneous determination of nine water and fat soluble vitamins after SPE separation and RP-HPLC analysis in pharmaceutical preparations and biological fluids, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 3203–3231.

SAMPLE
Matrix: cheese
Sample preparation: 500 mg Cheese + 2 mL 60% KOH + 2 mL 95% EtOH + 1 mL 1% NaCl + 5 mL 6% pyrogallol in EtOH, flush tube with nitrogen, seal, heat at 70° for 30 min, cool in ice water, add 15 mL 1% NaCl, extract twice with 15 mL portions of n-hexane:ethyl acetate 90:10. Combine the organic layers and evaporate them to dryness, dissolve the residue in 2 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere Si
Mobile phase: Gradient. A was n-hexane:isopropanol 99:1. B was n-hexane. A:B 50:50 for 7 min; to 90:10 over 4 min, maintain at 90:10 for 7 min, to 50:50 over 1 min, maintain at 50:50 for 4 min. (About every 100 injections recondition column with 50 mL dichloromethane, 50 mL isopropanol, and 50 mL dichloromethane.)
Flow rate: 1.5
Injection volume: 20
Detector: F ex 280 em 475 (vitamin E), UV 450 (β-carotene), and F ex 325 em 475 for 3.5 min, ex 280 em 475 for 10.5 min, ex 325 em 475 for 9 min (others)

CHROMATOGRAM
Retention time: 4.5
Limit of detection: 0.90 ng

OTHER SUBSTANCES
Extracted: β-carotene, vitamin A (all-trans-retinol), β-tocopherol, gamma-tocopherol, δ-tocopherol, 13-cis-retinol

KEY WORDS
normal phase; cheese

REFERENCE
Panfili,G.; Manzi,P.; Pizzoferrato,L. High-performance liquid chromatographic method for the simultaneous determination of tocopherols, carotenes, and retinol and its geometric isomers in Italian cheeses, *Analyst*, **1994**, *119*, 1161–1165.

SAMPLE
Matrix: food
Sample preparation: Melt 5 g margarine at 40° and homogenize it. Mix 20 g infant formula with 20 g water at 50°. Cut 5 g broccoli, mix in a homogenizer, freeze dry for 48 h at -40°, grind to a fine powder, mix 5 g in 30 mL water. Add 25 mL 600 g/L KOH to 5 g sample. Add solid KOH to the suspension until the KOH concentration is 60% (w/v). Add 15 mL EtOH to each 5 mL portion, shake. Add 25 mg ascorbic acid per gram, mix, flush with nitrogen, heat at 80° for 40 min. Immediately cool to room temperature. Add water to make EtOH:water ratio 0.3. Add 100 mL n-hexane:ethyl acetate 90:10, shake, let layers separate. Extract the aqueous phase with two 100 mL portions of n-hexane:ethyl acetate 90:10. Combine organic phases, wash with 100 ml water until the washes are neutral. Filter the organic phase through phase separating filter paper (S&S 597 HY, Schliecher and Schuell, Germany). Evaporate the filtrate under reduced pressure, dissolve the residue in hexane containing 20 mg/L 2,6-di-tert-butyl-4-methylphenol, inject a 30 mL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Lichrosorb Diol
Mobile phase: n-Hexane:MTBE 94:6
Column temperature: 23
Flow rate: 1
Injection volume: 30
Detector: F ex 295 em 330

CHROMATOGRAM
Retention time: 11.5 (α), 18.5 (β), 21.0 (γ), 30.5 (δ)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Extracted: 2-tert-butyl-4-hydroxyanisole, di-tert-butyl-4-methylphenol, plastochromanol-8, tocodienol, tocomoneols, tocotrienols

KEY WORDS
broccoli; formula; margarine

REFERENCE
Konings,E.J.M.; Roomans,H.H.S.; Beljaars,P.R. Liquid chromatographic determination of tocopherols and tocotrienols in margarine, infant foods, and vegetables, *J.AOAC Int.*, **1996**, *79*, 902–906.

SAMPLE
Matrix: food
Sample preparation: 500 mg Cheese + 2 mL 60% KOH + 2 mL 95% EtOH + 1 mL 1% NaCl + 5 mL 6% pyrogallol in EtOH, flush tube with nitrogen, seal, heat at 70, for 30 min, cool in ice water, add 15 mL 1% NaCl, extract twice with 15 mL portions of n-hexane:ethyl acetate 90:10 (Analyst 1994, 119, 1161). Combine the organic layers and evaporate them to dryness, dissolve the residue in MeOH:dichloromethane 90:10, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Supelco C18
Mobile phase: MeOH
Flow rate: 2
Detector: F ex 280 em 325

CHROMATOGRAM
Retention time: 4.2 (δ-tocopherol), 4.7 (β and γ-tocopherol), 5.3 (α-tocopherol)
Limit of detection: 1 ng

OTHER SUBSTANCES
Extracted: vitamin A (F ex 325 em 425), sterols (UV 208), carotenes (UV 450)

KEY WORDS
cheese

REFERENCE
Manzi,P.; Panfili,G.; Pizzoferrato,L. Normal and reversed-phase HPLC for more complete evaluation of tocopherols, retinols, carotenes and sterols in dairy products, *Chromatographia*, **1996**, *43*, 89–91.

SAMPLE
Matrix: food
Sample preparation: 500 mg Cheese + 2 mL 60% KOH + 2 mL 95% EtOH + 1 mL 1% NaCl + 5 mL 6% pyrogallol in EtOH, flush tube with nitrogen, seal, heat at 70, for 30 min, cool in ice water, add 15 mL 1% NaCl, extract twice with 15 mL portions of n-hexane:ethyl acetate 90:10 (Analyst 1994, 119, 1161). Combine the organic layers and evaporate them to dryness, dissolve the residue in n-hexane:2-propanol 99:1, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere Si (Beckman)
Mobile phase: Gradient. A was n-hexane:isopropanol 99:1. B was n-hexane. A:B 50:50 for 7 min; to 90:10 over 4 min, maintain at 90:10 for 7 min, to 50:50 over 1 min, maintain at 50:50 for 4 min. (About every 100 injections recondition column with 50 mL dichloromethane, 50 mL isopropanol, and 50 mL dichloromethane.)
Flow rate: 1.5
Detector: F ex 325 em 475

CHROMATOGRAM
Retention time: 4.5 (α-tocopherol), 8.0 (β-tocopherol), 8.7 (γ-tocopherol), 13.5 (δ-tocopherol)
Limit of detection: 900 pg (α), 800 pg (β), 500 pg (γ), 700 pg (δ)

OTHER SUBSTANCES
Extracted: vitamin A (13-cis-retinol, all trans-retinols) (F ex 325 em 425), sterols (UV 208), carotenes (UV 450)

KEY WORDS
cheese; normal phase

REFERENCE
Manzi,P.; Panfili,G.; Pizzoferrato,L. Normal and reversed-phase HPLC for more complete evaluation of tocopherols, retinols, carotenes and sterols in dairy products, *Chromatographia*, **1996**, *43*, 89–91.

SAMPLE
Matrix: formula
Sample preparation: Reconstitute 28 g milk based infant formula with ca. 145 g 78-80° water, mix thoroughly. Add 15 mL boiling isopropanol to 6.5 g reconstituted infant formula, mix thoroughly. Add 7.5 g magnesium sulfate, 30 mL hexane:ethyl acetate 85:15 and 1 mL BHT. Homogenize mixture for 1 min, filter through a coarse porosity glass filter by vacuum, wash the magnesium sulfate cake with two 15 mL portions of hexane:ethyl acetate 85:15. Repeat the extraction with 5 mL isopropanol and 15 mL hexane:ethyl acetate 85:15. Add 1 g magnesium sulfate to the combined filtrate and evaporate it to dryness under nitrogen. Dissolve the residue

in 10 mL hexane, filter (0.45 μm), evaporate to a volume of less than 5 mL at 45°. Dilute to 10 mL with mobile phase. Inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Lichrosorb Si 60
Mobile phase: Hexane:isopropanol 99.5:0.5
Flow rate: 1
Injection volume: 50
Detector: F ex 285 em 310

CHROMATOGRAM
Retention time: 2.7 (α-tocopheryl acetate), 10.1 (γ tocopherol), 17.3 (δ tocopherol)
Limit of detection: 3.14 ng/mL

KEY WORDS
infant formula; normal phase

REFERENCE
Chase,Jr.,G.W.; Eitenmiller,R.R.; Long,A.R. Liquid chromatographic analysis of all-RAC-α-tocopheryl acetate, tocopherols, and retinyl palmitate in SRM 1846, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 3317–3327.

SAMPLE
Matrix: formula, reference material
Sample preparation: 10 g Zero-control reference material (ZRM) powder + 50 g hot water, mix. 6.5 g Reconstituted ZRM or 3.5 g concentrated commercial formula + 10 mL boiling isopropanol, mix, add 7.5 g anhydrous magnesium sulfate to the ZRM and 4 g to the concentrated commercial formula, mix thoroughly, add 25 mL hexane:ethyl acetate 85:15, add 1 mL 360 μg/mL BHT, mix, homogenize (Polytron) for 1 min, filter through 60 mL coarse-porosity fritted glass filter using vacuum, wash with two 15 mL portions of hexane:ethyl acetate 85:15. Re-extract with 20 mL hexane:ethyl acetate 85:15 and 5 mL isopropyl alcohol, homogenize for 1 min, filter through 60 mL coarse-porosity fritted glass filter using vacuum, wash with two 15 mL portions of hexane:ethyl acetate 85:15. Mix the combined filtrate with 500 mg anhydrous magnesium sulfate, evaporate to dryness, add 15 mL hexane to the residue, filter (0.45 μm nylon) using vacuum, wash with three 7 mL portions of hexane, evaporate to 1 mL with nitrogen at 45°, dilute to 10 mL with hexane, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Lichrosorb Si 60
Mobile phase: Hexane:isopropanol 99.5:0.5
Flow rate: 1
Injection volume: 50
Detector: F ex 285 em 310

CHROMATOGRAM
Retention time: 3.4 (vitamin E acetate)
Limit of quantitation: 2.0 μg/mL

OTHER SUBSTANCES
Simultaneous: (R,R,R)-α.-tocopherol, δ-tocopherol, γ -tocopherol
Also analyzed: vitamin A

KEY WORDS
normal phase; soy-based infant formula

REFERENCE
Chase,G.W.,Jr.; Long,A.R.; Eitenmiller,R.R. Liquid chromatographic method for analysis of all-rac-α-tocopheryl acetate and retinyl palmitate in soy-based infant formula using a zero-control reference material (ZRM) as a method development tool, *J.AOAC Int.*, **1998**, *81*, 577–581.

SAMPLE
Matrix: formulations

Sample preparation: Mix 100-150 mg of the formulation with celite and extract with supercritical carbon dioxide at 250 atmospheres at 40° at 190-220 mL/min with the restrictor at 100° (Dionex SFE-703), collect in 4 mL THF:MeOH 80:20 at 0°, make up to 5 mL, inject an aliquot.

HPLC VARIABLES
Guard column: 10 μm Guard-Pak (Waters)
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:MeOH 25:75
Flow rate: 1.5
Detector: UV 280

CHROMATOGRAM
Retention time: 6 (vitamin E acetate)

OTHER SUBSTANCES
Simultaneous: vitamin A palmitate (UV 325)

KEY WORDS
SFE; cream; lotion; protect from light

REFERENCE
Scalia,S.; Renda,A.; Ruberto,G.; Bonina,F.; Menegatti,E. Assay of vitamin A palmitate and vitamin E acetate in cosmetic creams and lotions by supercritical fluid extraction and HPLC, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 273–277.

SAMPLE
Matrix: formulations
Sample preparation: Grind tablets to a fine powder, weigh out 100-150 mg, mix with sea sand. Extract with supercritical carbon dioxide (Dionex) in the dynamic mode at 250 atmospheres and 40° for 15 min (restrictor 60°, gaseous flow rate 190-220 mL/min), collect in 6 mL THF at 0°, make up to 10 mL with THF, inject an aliquot. [Alternatively, add 5 crushed tablets or the contents from 5 capsules to 10 mL DMSO, add 15 mL hexane, shake at 60° for 45 min, centrifuge at 3000 rpm for 10 min, remove hexane layer, add 15 mL hexane, vortex for 5 min at room temperature, remove hexane layer, repeat hexane extraction three more times, combine all hexane layers, dilute with THF, inject a 25 μL aliquot. J.Assoc.Off.Anal.Chem. 1989, 72, 247.]

HPLC VARIABLES
Guard column: 4 × 4 5 μm (Merck)
Column: 250 × 4 5 μm Lichrospher CH-8
Mobile phase: MeOH:MeCN 75:25
Flow rate: 1.3
Injection volume: 50
Detector: UV 280

CHROMATOGRAM
Retention time: 6 (vitamin E acetate)

OTHER SUBSTANCES
Simultaneous: vitamin A palmitate (UV 325)

KEY WORDS
tablets; SFE

REFERENCE
Scalia,S.; Ruberto,G.; Bonina,F. Determination of vitamin A, vitamin E, and their esters in, *J.Pharm.Sci.*, **1995**, *84*, 433–436.

SAMPLE
Matrix: fat spread products, margarine

Sample preparation: Melt two sticks of margarine at 45°. Homogenize spreads without warming. Weigh 5.0 g sample. Add 40 mL hexane-BHT and sonicate with intermittent mixing. Rinse side with 10 mL hexane-BHT, add 3 drops of Tween 80, 3 g anhydrous magnesium sulfate (1g for each mL of water content/1g magnesium sulfate) and vortex. Stand for ≥ 2 h. Filter by medium porosity fritted glass filter. Wash with hexane-BHT. Filtrate dilute to 100 mL with hexane-BHT. Pipet 1.0 mL and dilute to 50 mL with hexane-BHT. Inject an 20 μL aliquot.

HPLC VARIABLES
Guard column: 5 μm LiChrosorb Si 60
Column: 250 × 4.6 5 μm LiChrosorb Si 60
Mobile phase: n-Hexane:isopropyl alcohol 99.1:0.9
Flow rate: 1.0
Injection volume: 20
Detector: F ex 290 em 330

CHROMATOGRAM
Retention time: 10.6
Limit of detection: 1.98 μg/100g
Limit of quantitation: 3.02 μg/100g

OTHER SUBSTANCES
Extracted: α-tocopherol, γ-tocopherol

KEY WORDS
δ-tocopherol

REFERENCE
Ye,L.; Landen,W.O.,Jr.; Lee,J.; Eitenmiller,R.R. Vitamin E content of margarine and reduced fat products using a simplified extraction procedure and HPLC determination, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 1227–1238.

SAMPLE
Matrix: fat spread products, margarine
Sample preparation: Melt two sticks of margarine at 45°. Homogenize spreads without warming. Weigh 5.0 g sample. Add 40 mL hexane-BHT and sonicate with intermittent mixing. Rinse side with 10 mL hexane-BHT, add 3 drops of Tween 80, 3 g anhydrous magnesium sulfate (1g for each mL of water content/1g magnesium sulfate) and vortex. Stand for ≥ 2 h. Filter by medium porosity fritted glass filter. Wash with hexane-BHT. Filtrate dilute to 100 mL with hexane-BHT. Pipet 1.0 mL and dilute to 50 mL with hexane-BHT. Inject an 20 μL aliquot.

HPLC VARIABLES
Guard column: 5 μm LiChrosorb Si 60
Column: 250 × 4.6 5 μm LiChrosorb Si 60
Mobile phase: n-Hexane:isopropyl alcohol 99.1:0.9
Flow rate: 1.0
Injection volume: 20
Detector: F ex 290 em 330

CHROMATOGRAM
Retention time: 7.5
Limit of detection: 2.96 μg/100g
Limit of quantitation: 5.00 μg/100g

OTHER SUBSTANCES
Extracted: α-tocopherol, δ-tocopherol

KEY WORDS
γ-tocopherol

REFERENCE

Ye,L.; Landen,W.O.,Jr.; Lee,J.; Eitenmiller,R.R. Vitamin E content of margarine and reduced fat products using a simplified extraction procedure and HPLC determination, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 1227–1238.

SAMPLE

Matrix: margarine, spreads

Sample preparation: Sonicate 5.0 g homogenized margarine or spread with 40 mL 0.1% BHT in hexane with intermittent mixing until sample has dissolved. Rinse sides of flask with 10 mL 0.1% BHT in hexane, add 3 drops Tween 80, add 3 g anhydrous magnesium sulfate (1 g for each 1 mL of water content plus 1 g extra), mix, let stand for ≥ 2 h. Filter (medium porosity fritted glass filter) and wash the filter with 0.1% BHT in hexane, dilute the filtrate to 100 mL with 0.1% BHT in hexane. Dilute a 1.0 mL aliquot to 50 mL with 0.1% BHT in hexane, filter (0.45 μ m). Inject an 20 μL aliquot.

HPLC VARIABLES

Guard column: 5 μm LiChrosorb Si 60
Column: 250 × 4.6 5 μm LiChrosorb Si 60
Mobile phase: n-Hexane:isopropyl alcohol 99.1:0.9
Flow rate: 1.0
Injection volume: 20
Detector: F ex 290 em 330

CHROMATOGRAM

Retention time: 4.8
Limit of detection: 232 ng/g
Limit of quantitation: 398.4 ng/g

OTHER SUBSTANCES

Extracted: γ-tocopherol, δ-tocopherol

KEY WORDS

normal phase

REFERENCE

Ye,L.; Landen,W.O.,Jr.; Lee,J.; Eitenmiller,R.R. Vitamin E content of margarine and reduced fat products using a simplified extraction procedure and HPLC determination, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 1227–1238.

SAMPLE

Matrix: milk

Sample preparation: Mix 50 mL milk with 30 mL ethanolic KOH (10:30). Saponify the mixture at 80° for 20min. Extract twice with 10 mL n-hexane. Evaporate to dryness, reconstitute the residue with 1 mL mobile phase, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5μm C18 (Alltech)
Mobile phase: MeOH:EtOH 80:20 (A) or EtOH:water 95:5 (B)
Flow rate: 1
Injection volume: 5
Detector: UV 250

CHROMATOGRAM

Retention time: 8.9 (A), 11 (B)

OTHER SUBSTANCES

Extracted: isotretinoin, retinal, tretinoin, vitamin A, vitamin D2, vitamin D3, vitamin K1, vitamin K2

REFERENCE

Gong,B.Y.; Ho,J.W. Simultaneous separation and detection of ten common fat-soluble vitamins in milk, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2389–2397.

SAMPLE
Matrix: milk
Sample preparation: Add 500 mg ascorbic acid, 50 mL EtOH, and 10 mL 60% KOH to 25 g liquid or reconstituted powdered milk under a nitrogen stream, stir overnight at room temperature. Extract with three 50 mL portions of n-hexane and two 25 mL portions of n-hexane by shaking for 2 min each. Combine the n-hexane extracts, wash with 50 mL portions of water containing a few drops of phenolphthalein until the aqueous phase appears colorless, add 1 g butylated hydroxytoluene, filter through a Whatman No.1 filter containing 20 g anhydrous sodium sulfate, concentrate the filtrate under reduced pressure at 40°, reconstitute with 10 mL MeOH, filter (0.45 μm), inject an aliquot of the filtrate.

HPLC VARIABLES
Guard column: Tracer Spherisorb ODS 2 C18
Column: 250 × 4.6 5 μm Tracer Spherisorb ODS 2 C18 (Teknokroma, Spain)
Mobile phase: MeCN:MeOH:water 1:95:4
Injection volume: 20
Detector: UV 323 for 14 min then UV 292

CHROMATOGRAM
Retention time: 20
Limit of detection: 300 ng/mL
Limit of quantitation: 400 ng/mL

OTHER SUBSTANCES
Extracted: vitamin A

REFERENCE
Albalá-Hurtado,S.; Novella-Rodríguez,S.; Veciana-Nogués,M.; Mariné-Font,A. Determination of vitamins A and E in infant milk formulae by high-performance liquid chromatography, *J.Chromatogr.A*, **1997**, *778*, 243–246.

SAMPLE
Matrix: milk
Sample preparation: 1 g Powdered milk or 25 mL liquid milk + alcoholic KOH, let stand overnight, extract with hexane. Remove the organic layer and evaporate it to dryness, reconstitute the residue in MeOH, filter, inject an aliquot. (Alcoholic KOH was prepared from 50 mL EtOH and 15 mL 60% KOH in water.)

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm RP18 (Brownlee)
Column: 220 × 4.6 5 μm OD-224 RP18 (Brownlee)
Mobile phase: MeOH:water 99:1 containing 2.5 mM acetic acid/sodium acetate
Flow rate: 1.25
Injection volume: 10
Detector: E, EG & G PAR Model 400, MP 1304 glassy carbon series dual electrode, E1 (upstream) -1100 mV, E2 (downstream) +700 mV (Condition electrodes for 30 min at E1 -1200 mV and E2 +1500 mV at the start of each day.)

CHROMATOGRAM
Retention time: 9
Limit of detection: 0.19 ng

OTHER SUBSTANCES
Extracted: vitamin A

REFERENCE
Delgado-Zamarreño,M.M.; Sanchez Perez,A.; Gomez Perez,M.C.; Fernandez Moro,M.A.; Hernandez Mendez,J. Determination of vitamins A, E and K1 in milk by high-performance liquid chromatography with dual amperometric detection, *Analyst*, **1995**, *120*, 2489–2492.

SAMPLE
Matrix: milk

Sample preparation: Dilute milk to 30% with water, mix with reagent and pass through a 5 m × 0.5 mm i.d. PTFE tube knotted reactor at 1.25 mL/min, mix with 2.5 M acetic acid, pass onto column a Sep-Pak Plus C18 SPE cartridge for 5 min, wash column A with MeOH:water 40:60 for 4 min, elute column A with MeOH for 4 min, inject the last 100 μL aliquot of the eluate. (Reagent was 50 mL EtOH + 15 mL 60% aqueous NaOH + 5 mL 10% ascorbic acid.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm RP18 (Brownlee)
Column: 220 × 4.6 5 μm OD-224 RP18 (Brownlee)
Mobile phase: MeOH:water 99:1 containing 2.5 mM acetic acid-sodium acetate
Flow rate: 1
Injection volume: 100
Detector: E, glassy carbon working electrode +1300 mV

CHROMATOGRAM
Retention time: 12
Limit of detection: 311 nM

OTHER SUBSTANCES
Extracted: vitamin A, vitamin D3

KEY WORDS
SPE

REFERENCE
Delgado-Zamarreño,M.M.; Sanchez-Perez,A.; Gomez-Perez,M.C.; Hernandez-Mendez,J. Directly coupled sample treatment-high-performance liquid chromatography for on-line automatic determination of liposoluble vitamins in milk, *J.Chromatogr.A*, **1995**, *694*, 399–406.

SAMPLE
Matrix: silicone oils
Sample preparation: Condition a 1 g Si Bond-Elut SPE cartridge with 5 mL n-hexane. Mix 1 g silicone oil with 2 mL dichloromethane, vortex for 2 min, centrifuge at 3000 g. Withdrawn the supernatant, repeat this procedure twice, filter (0.45 μm), heat the filtrate at 50°, expose to a stream of helium for 30 min. Add 2.5 μg retinol acetate, 2.5 μg α-tocopherol acetate, and 25 μg BHT. Add the mixture to the SPE cartridge, elute with 500 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax C8
Mobile phase: Gradient. A was MeCN:200 mM ammonium acetate 72:25. B was MeOH:water 95:5. A:B 100:0 for 10 min, to 0:100 over 1 min, maintain at 0:100 for 14 min
Flow rate: 2 for 10 min then 1.5
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 16
Internal standard: α-tocopherol acetate (18)
Limit of detection: 604.9 ng/mL
Limit of quantitation: 2.016 μg/mL

OTHER SUBSTANCES
Extracted: cholesterol (UV 210), retinal (UV 350), retinoic acid (UV 350), retinol acetate (UV 350), vitamin A (UV 350)

KEY WORDS
ophthalmic silicone oils; SPE

REFERENCE
Del Nozal,M.J.; Bernal,J.L.; Marinero,P. Simultaneous HPLC determination of cholesterol, α-tocopherol, retinol, retinal and retinoic acid in silicone oils used as vitreous substitutes in eye surgery, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1151–1167.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 8 μm Unisphere-PBD (polybutadiene on alumina) (Biotage, Charlottesville, VA)
Mobile phase: MeOH:water 92:8
Flow rate: 1
Detector: UV 330

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: retinoic acid, retinol acetate, vitamin A

REFERENCE
Jedrejewski,P.T.; Taylor,L.T. Comparison of silica-, alumina-, and polymer-based stationary phases for reversed-phase liquid chromatography, *J.Chromatogr.Sci.*, **1995**, *33*, 438–445.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 3 μm Adsorbosphere-HS C18
Mobile phase: MeCN:isopropanol:MeOH 60:30:10 containing 0.1% ammonium acetate
Flow rate: 1
Detector: UV 234, UV 295, UV 450

OTHER SUBSTANCES
Simultaneous: β-carotene

REFERENCE
Maitra,I.; Marcocci,L.; Droy-Lefaix,M.T.; Packer,L. Peroxyl radical scavenging activity of *Gingko biloba* extract EGb 761, *Biochem.Pharmacol.*, **1995**, *49*, 1649–1655.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 100 μg/mL solution in hexane.

HPLC VARIABLES
Guard column: 15 × 3.2 silica (Applied Biosystems)
Column: 300 × 3.9 μBondapak NH2 aminopropylmethylsilyl bonded silica
Mobile phase: Cyclohexane:MTBE 90:10
Flow rate: 1
Injection volume: 10
Detector: F ex 298 em 345

CHROMATOGRAM
Retention time: 16

OTHER SUBSTANCES
Simultaneous: β-tocopherol, gamma-tocopherol, δ-tocopherol

KEY WORDS
normal phase; discussion of other columns and mobile phases

REFERENCE
Abidi,S.L.; Mounts,T.L. Normal phase high-performance liquid chromatography of tocopherols on polar phases, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 509–520.

SAMPLE
Matrix: tissue
Sample preparation: Add aliquots of 1.6 nM IS to samples, resuspend them by sonication in 300 μL 1% ascorbate in 100 mM sodium dodecyl sulphate and 450 μL absolute EtOH, extract once with 800 μL hexane, mix for 30 s. Evaporate hexane extracts to dryness with nitrogen, resuspend in 1 mL MeOH containing 2.5% ascorbate. Inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb ODS II
Mobile phase: MeOH:water 96:4
Flow rate: 1.8
Injection volume: 100
Detector: F ex 210 em 300

CHROMATOGRAM
Retention time: 8.0
Internal standard: δ-tocopherol

OTHER SUBSTANCES
Extracted: α-tocopherol, α-tocopheryloxybutyric acid

KEY WORDS
rat; liver; human; leukemia cell; δ-tocopherol is IS

REFERENCE
Tirmenstein,M.A.; Watson,B.W.; Haar,N.C.; Fariss,M.W. Sensitive method for measuring tissue α-tocopherol and α-tocopheryloxybutyric acid by high-performance liquid chromatography with fluorometric detection, *J.Chromatogr.B*, **1998**, *707*, 308–311.

SAMPLE
Matrix: tissue
Sample preparation: Add aliquots of 1.6 nmoles IS to tissue samples, resuspend by sonication in 300 μL 1% ascorbate in 100 mM sodium dodecyl sulfate and 450 μL absolute EtOH, extract once with 800 μL hexane, mix for 30 s. Evaporate hexane extracts to dryness with nitrogen, resuspend in 1 mL MeOH containing 2.5% ascorbate. Inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb ODS II
Mobile phase: MeOH:water 96:4
Flow rate: 1.8
Injection volume: 100
Detector: F ex 210 em 300

CHROMATOGRAM
Retention time: 11.5
Internal standard: d-δ-tocopherol (Henkel) (8.0)
Limit of detection: 3 pmoles
Limit of quantitation: 6 pmoles

OTHER SUBSTANCES
Extracted: α-tocopheryloxybutyric acid

KEY WORDS
rat; liver; human; leukemia cell

REFERENCE
Tirmenstein,M.A.; Watson,B.W.; Haar,N.C.; Fariss,M.W. Sensitive method for measuring tissue α-tocopherol and α-tocopheryloxybutyric acid by high-performance liquid chromatography with fluorometric detection, *J.Chromatogr.B*, **1998**, *707*, 308–311.

SAMPLE
Matrix: tissue
Sample preparation: 50 mg Tissue + 50 μL 560 μg/mL vitamin K in EtOH, extract twice with 1 mL n-hexane using a sonicator, centrifuge at 2000 g for 5 min. Evaporate the supernatant to dryness, reconstitute it in 200 μL chloroform:MeOH 25:75, inject an aliquot. (Caution! Chloroform is a carcinogen!)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Nucleosil 120 C18
Mobile phase: MeOH:water 96.5:3.5
Column temperature: 40
Flow rate: 2
Detector: F ex 295 em 350

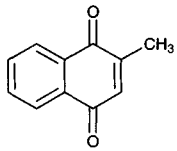
CHROMATOGRAM
Retention time: ca. 5
Internal standard: vitamin K (9)

OTHER SUBSTANCES
Extracted: vitamin A (UV 325), vitamin A_p (UV 325)

KEY WORDS
rat; liver; placenta; brain

REFERENCE
Barbas,C.; Castro,M.; Bonet,B.; Viana,M.; Herrera,E. Simultaneous determination of vitamins A and E in rat tissues by high-performance liquid chromatography, *J.Chromatogr.A*, **1997**, *778*, 415–420.

Vitamin K



CAS Registry No.: 83-70-5, 523-68-2 (N-acetyl analog), 130-24-5 (K_5 HCl), 84-80-0 (K_1), 25486-55-9 (K_1 oxide), 84-81-1 ($K_{2(30)}$), 58-27-5 (K_3)
Merck Index: 10161

SAMPLE
Matrix: milk
Sample preparation: Mix 50 mL milk with 30 mL ethanolic KOH (10:30). Saponify the mixture at 80° for 20min. Extract twice with 10 mL n-hexane. Evaporate the extracts to dryness, reconstitute the residue with 1 mL mobile phase, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5μm C18 (Alltech)
Mobile phase: MeOH:EtOH 80:20 (A) or EtOH:water 95:5 (B)
Flow rate: 1
Injection volume: 5
Detector: UV 250

CHROMATOGRAM
Retention time: 10.8 (K1 (?), A), 16.7 (K2 (?), A), 12 (K1 (?), B), 20 (K2 (?), B)

OTHER SUBSTANCES
Extracted: isotretinoin, retinal, tretinoin, vitamin A, vitamin D2, vitamin D3, vitamin E

REFERENCE
Gong,B.Y.; Ho,J.W. Simultaneous separation and detection of ten common fat-soluble vitamins in milk, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2389–2397.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a solution in chloroform:MeOH 25:75. (Caution! Chloroform is a carcinogen!)

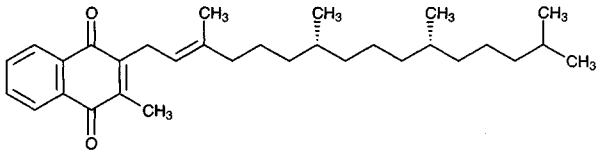
HPLC VARIABLES
Column: 150 × 4.6 5 μm Nucleosil 120 C18
Mobile phase: MeOH:water 96.5:3.5
Column temperature: 40
Flow rate: 2
Detector: UV 325, F ex 295 em 350

CHROMATOGRAM
Retention time: 9

OTHER SUBSTANCES
Simultaneous: vitamin A, vitamin A_p, vitamin E

REFERENCE
Barbas,C.; Castro,M.; Bonet,B.; Viana,M.; Herrera,E. Simultaneous determination of vitamins A and E in rat tissues by high-performance liquid chromatography, *J.Chromatogr.A*, **1997**, *778*, 415–420.

Vitamin K1



Molecular formula: $C_{31}H_{46}O_2$
Molecular weight: 450.71
CAS Registry No.: 84-80-0
Merck Index: 7536

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 50 ng IS + 1 mL 0.9% NaCl + 3 mL isopropanol + 10 mL n-hexane, mix by inversion at 20 rpm for 1 h, centrifuge at 1000 g for 10 min. Remove the upper layer and evaporate it to dryness under reduced pressure at room temperature, reconstitute the residue in 1 mL MeOH, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 100 × 3 5 μm Hypersil MOS
Mobile phase: MeOH:water 92.5:7.5 containing 30 mM sodium chlorate (Continuously bubble oxygen-free nitrogen (presaturated with mobile phase) through the mobile phase reservoir.)
Flow rate: 0.9
Injection volume: 50
Detector: F ex 320 em 420 following post-column reaction. The mobile phase flowed through an electrochemical cell with coulometric reduction at -400 mV to the detector.

CHROMATOGRAM
Retention time: 10
Internal standard: vitamin $K_{2(30)}$ (17)
Limit of quantitation: 1 ng/mL

KEY WORDS
post-column reaction; plasma; protect from light

REFERENCE

Langenberg,J.P.; Tjaden,U.R. Determination of (endogenous) vitamin K_1 in human plasma by reversed-phase high-performance liquid chromatography using fluorometric detection after post-column electrochemical reduction. Comparison with ultraviolet, single and dual electrochemical detection, *J.Chromatogr.*, **1984**, *305*, 61–72.

SAMPLE

Matrix: blood

Sample preparation: 0.5-1 mL Plasma + 20 μL 87.5 ng/mL IS in EtOH + 2 mL EtOH + 6 mL hexane, mix, centrifuge at 3500 g for 5 min. Remove the upper hexane layer and evaporate it to dryness at 30° in a vortex-type evaporator, reconstitute the residue in hexane, add to a Sep-Pak silica SPE cartridge, wash with 8 mL hexane, elute with 8 mL hexane:diethyl ether 97:3, evaporate the eluate to dryness in a vortex-type evaporator, reconstitute with 1 mL hexane, add 4 mL reagent, add 5-10 mg zinc metal, vortex for 2 min, centrifuge, discard the hexane layer. Remove the lower (MeCN) layer and evaporate it to dryness, reconstitute the residue in 6 mL hexane and 2 mL water, vortex, centrifuge. Remove the upper hexane layer and evaporate it to dryness under a stream of air at 60°, reconstitute the residue in 250 μL mobile phase, inject a 100 μL aliquot. (Reagent was 70 mM zinc chloride in MeCN:acetic 97:3.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Hypersil-ODS

Mobile phase: MeOH:dichloromethane:buffer 80:20:0.5 (Buffer was 2 M zinc chloride containing 1 M sodium acetate and 1 M acetic acid.)

Flow rate: 1

Injection volume: 100

Detector: F ex 248 em 418 (longpass cut-off filter) following post-column reaction. The column effluent flowed through a 20 × 3.9 column packed with 200 mesh zinc particles (to remove oxygen and reduce the vitamin K) to the detector.

CHROMATOGRAM

Retention time: 9.5

Internal standard: dihydro-vitamin K_1 (10.5)

Limit of detection: 50 pg/mL

OTHER SUBSTANCES

Extracted: vitamin K_1 epoxide

KEY WORDS

plasma; SPE; post-column reaction; pharmacokinetics

REFERENCE

Haroon,Y.; Bacon,D.S.; Sadowski,J.A. Liquid-chromatographic determination of vitamin K_1 in plasma, with fluorimetric detection, *Clin.Chem.*, **1986**, *32*, 1925–1929.

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 2 mL EtOH, vortex for 3 min, add 3 mL hexane, vortex for 5 min, centrifuge at 1500 g for 10 min, repeat extraction with 3 mL hexane. Combine the hexane layers and wash with 2 mL MeOH:water 9:1. Remove the upper organic layer and filter (0.45 μm) it, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 33.4 μL EtOH, inject a 5 μL aliquot.

HPLC VARIABLES

Guard column: ODS

Column: 125 × 4 5 μm LiChrospher 100 RP-18 or 100 × 2.1 3 μm Spherisorb ODS-2

Mobile phase: Gradient. A was MeOH:water 99:1. B was MeOH:THF 70:30. A:B 100:0 for 2 min, 5:95 for 3.5 min, 0:100 for 4.5 min, re-equilibrate for 2 min (step gradients).

Flow rate: 1.5 (125 × 4 column) or 0.2 (100 × 2.1 column)

Injection volume: 5

Detector: UV 250

CHROMATOGRAM
Retention time: 6.5 (125 × 4 column), 7.5 (100 × 2.1 column)
Limit of detection: 0.42 ng (100 × 2.1 column), 6.4 ng (125 × 4 column)

OTHER SUBSTANCES
Extracted: retinyl palmitate (UV 328), vitamin E (α-tocopherol) (UV 284), vitamin D3 (cholecalciferol) (UV 265)

KEY WORDS
cow; plasma; protect from light; degas stock solutions with helium; narrow bore

REFERENCE
Gomis,D.B.; Escotet Arias,V.J.; Fidalgo Alvarez,L.E.; Gutiérrez Alvarez,M.D. Simultaneous determination of vitamins D3, E and K1 and retinyl palmitate in cattle plasma by liquid chromatography with a narrow-bore column, *J.Chromatogr.B*, **1994**, *660*, 49–55.

SAMPLE
Matrix: blood
Sample preparation: Condition a 500 mg Enviroprep Inert silica SPE cartridge (Baxter) with 5 mL hexane. 500 μL Plasma + 1 mL 1 ng/mL IS in isopropanol, vortex for 5 s, add 2 mL hexane, vortex for 30 s, centrifuge at 2000 g for 5 min, remove the hexane layer, repeat extraction twice more. Combine the organic layers and evaporate them to dryness under a stream of nitrogen (make sure all alcohol is removed), reconstitute the residue in 500 μL hexane, vortex thoroughly, add to the SPE cartridge, wash with 10 mL hexane, elute with 5 mL hexane: ether 97:3, discard the first 1 mL, evaporate the next 4 mL eluate to dryness under a stream of nitrogen, reconstitute with 100-500 μL EtOH, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm 201TP 54 (Vydac)
Mobile phase: EtOH:MeOH 40:60
Flow rate: 1
Injection volume: 50
Detector: F ex 242 em 430 (or 280 cut-off filter) following post-column reaction. The column effluent flowed through a 50 × 4 column packed with 10% Pt on alumina catalyst (Alfa) to the detector. (Caution! Catalyst/flammable solvent mixtures may ignite!)

CHROMATOGRAM
Retention time: 11
Internal standard: vitamin K_2 (menaquinone-4) (8)
Limit of detection: 20 pg/mL

KEY WORDS
serum; post-column reaction; SPE

REFERENCE
MacCrehan,W.A.; Schönberger,E. Determination of vitamin K1 in serum using catalytic-reduction liquid chromatography with fluorescence detection, *J.Chromatogr.B*, **1995**, *670*, 209–217.

SAMPLE
Matrix: formula
Sample preparation: 20 mL Infant formula + 4 mL concentrated ammonium hydroxide, swirl for 1 min, add 60 mL MeOH, swirl for 30 s, add 100 mL dichloromethane, add 50 mL isooctane, shake well, repeat extraction. Combine the organic layers and evaporate them to dryness under reduced pressure at <75°, reconstitute the residue in 20 mL acetone, evaporate, repeat acetone evaporation twice, flush flask with nitrogen, reconstitute with 10 mL isooctane:isopropanol 99.99:0.01, add to silica column, rinse flask with two 5 mL portions of isooctane:isopropanol 99.99:0.01, add rinses to column, elute column until solvent is 5 mm below top of sodium sulfate layer, rinse column top with 10 mL isooctane:isopropanol 99.99:0.01, discard eluate, elute with 100 mL isooctane:dichloromethane:isopropanol 84.98:15:0.02 (volume may need to be determined experimentally), evaporate eluate to dryness under reduced pressure at <75°, reconstitute with 5 mL isooctane, inject a 40 μL aliquot. (Prepare silica column by adding 5 g 60-200

mesh silica (Mallinckrodt SiliCAR Grade 62 Special, dry at 100° overnight) and 2 g anhydrous sodium, sulfate (dried at 100° overnight) to a 300 × 10 glass column, add 20 mL isooctane: isopropanol 99.99:0.01, elute until top of solvent layer is 5 mm below top of sodium sulfate layer.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Apex I silica (Jones Chromatography)
Mobile phase: Isooctane:dichloromethane:isopropanol 69.98:30:0.02
Flow rate: 1
Injection volume: 40
Detector: UV 254

CHROMATOGRAM
Retention time: 6.7 (cis), 7.7 (trans)

KEY WORDS
protect from light; normal phase; SPE

REFERENCE
Tanner,J.T.; Barnett,S.A.; Mountford,M.K. Analysis of milk-based infant formula. Phase IV. Iodide, linoleic acid, and vitamins D and K: U.S. Food and Drug Administration-Infant Formula Council: Collaborative study, *J.AOAC Int.*, **1993**, *76*, 1042–1056.

SAMPLE
Matrix: formula, milk
Sample preparation: Dissolve 3 g infant formula or milk powder in 15 mL warm water with thorough mixing. 15 mL Ready-to-use formula or prepared solution + 5 mL buffer + 1 g lipase (Type VII from Candida cylindracea, 700-1500 U/mg, Sigma), shake mechanically for 5 min, heat at 37° with sonication for 2 h (shake vigorously every 20 min), cool, add 10 mL EtOH: MeOH 95:5, add 1 g potassium carbonate, add 1 mL 100 μg/mL cholesteryl phenylacetate in hexane, add 15 mL hexane, shake mechanically for 7 min, centrifuge for 5 min, repeat extraction with 15 mL hexane. Combine the organic layers and evaporate a 25 mL aliquot to near dryness under reduced pressure at 40°, reconstitute the residue in 100 μL hexane inject the whole amount into a 100 × 8 5 μm Resolve silica radial compression column (Waters) with a Guard-Pak silica precolumn (Waters) eluted with hexane:isopropanol 99.9:0.1 at 2 mL/min, monitor effluent at UV 269, collect the fraction between 2 and 4.5 min (then purge column at 8 mL/min for 30 min). Evaporate the fraction to dryness under a stream of nitrogen, reconstitute the residue in 200-500 μL isopropanol, inject a 20-50 μL aliquot. (Buffer was 800 mM KH_2PO_4 adjusted to pH 8.0 with NaOH.)

HPLC VARIABLES
Guard column: Guard-Pak C18
Column: 100 × 8 5 μm Resolve C18 radial compression (Waters)
Mobile phase: MeOH:isopropanol:ethyl acetate:water 45:35:14.5:13.5 (After IS elutes purge column with MeOH:ethyl acetate 50:50 for 10 min, re-equilibrate for 5 min.)
Flow rate: 2
Injection volume: 20-50
Detector: UV 269, UV 277

CHROMATOGRAM
Retention time: 26
Internal standard: cholesteryl phenylacetate (43)
Limit of detection: 5 ng/g
Limit of quantitation: 50 ng/mL

REFERENCE
Indyk,H.E.; Littlejohn,V.C.; Lawrence,R.J.; Woollard,D.C. Liquid chromatographic determination of vitamin K1 in infant formulas and milk, *J.AOAC Int.*, **1995**, *78*, 719–723.

SAMPLE
Matrix: milk

Sample preparation: 500 μL Milk + 50 μL 54 ng/mL IS in EtOH + 10 μL 200 mg/mL albumin + 200 μL water containing 50 mM sodium taurocholate, 100 mM calcium chloride, and 150 mM NaCl, sonicate (titanium probe MSE Scientific Instruments) for 2 min (30 s on, 30 s wait) in an ice bath, add 1.2 mL 3.4 mg/mL crude lipase (porcine pancreas Type II (EC 3.1.1.3), Sigma), in 200 mM pH 7.7 Tris buffer, shake at 37° at 100 strokes/min for 45 min, add 4 mL EtOH, add 2 mL water, add 200 μL 50 g/L ammonium hydroxide, add 7.5 mL n-hexane, vortex for 2 min, inject an aliquot of the hexane layer on to a 200 × 4.6 5 μm RSIL column (RSL, Eke, Belgium) and elute with n-hexane:diisopropyl ether 98.5:1.5 at 0.85 mL/min (Caution! Diisopropyl ether readily forms explosive peroxides!), collect the fraction containing vitamin K and the IS, evaporate to dryness, reconstitute with 75 μL MeOH:ethyl acetate 96:4, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.2 5 μm RoSIL HL (RSL, Eke, Belgium)
Mobile phase: MeOH:ethyl acetate 96:4 containing 2.25 g/L tetramethylammonium octahydridotriborate (Alfa)
Flow rate: 0.7
Injection volume: 50
Detector: F ex 325 em 430 following post-column reaction. The column effluent flowed through a 5 m × 0.5 mm i.d. knitted PTFE coil at 80° to the detector (the phylloquinone is reduced).

CHROMATOGRAM

Retention time: 7
Internal standard: a structural analog of phylloquinone with one more isoprene unit (Hoffman-La Roche) (11)
Limit of detection: 0.035 ng/mL
Limit of quantitation: 0.08 ng/mL

KEY WORDS

protect from light; post-column reaction

REFERENCE

Lambert,W.E.; Vanneste,L.; De Leenheer,A.P. Enzymatic sample hydrolysis and HPLC in a study of phylloquinone concentration in human milk, *Clin.Chem.*, **1992**, *38*, 1743–1748.

SAMPLE

Matrix: milk
Sample preparation: Condition a Sep-Pak silica SPE cartridge with hexane. Treat 25 (?) mL milk with 2.5 g lipase at 37° for 90 min, treat with alcoholic NaOH for 15 s, extract twice with 50 mL portions of hexane. Evaporate the hexane extract, reconstitute with 5 mL hexane, add to the SPE cartridge, wash with 4 mL hexane, elute with 4 mL hexane:ether 96:4, inject an aliquot of the eluate.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP18 (Brownlee)
Column: 220 × 4.6 5 μm OD-224 RP18 (Brownlee)
Mobile phase: MeOH:water 99:1 containing 2.5 mM acetic acid/sodium acetate
Flow rate: 1.25
Injection volume: 10
Detector: E, EG & G PAR Model 400, MP 1304 glassy carbon series dual electrode, E1 (upstream) -1100 mV, E2 (downstream) +700 mV (Condition electrodes for 30 min at E1 -1200 mV and E2 +1500 mV at the start of each day.)

CHROMATOGRAM

Retention time: 19
Limit of detection: 3.1 ng

OTHER SUBSTANCES

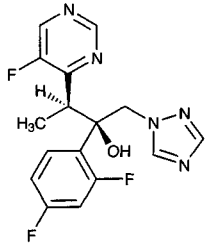
Noninterfering: vitamin A, vitamin E

KEY WORDS

SPE

REFERENCE
Delgado-Zamarreño,M.M.; Sanchez Perez,A.; Gomez Perez,M.C.; Fernandez Moro,M.A.; Hernandez Mendez,J. Determination of vitamins A, E and K1 in milk by high-performance liquid chromatography with dual amperometric detection, *Analyst*, **1995**, *120*, 2489–2492.

Voriconazole



Molecular formula: $C_{16}H_{14}F_3N_5O$
Molecular weight: 349.32
CAS Registry No.: 137234-62-9

SAMPLE

Matrix: blood
Sample preparation: Mix 700 μL plasma with 300 μL IS in 20 mM pH 6.5 ammonium phosphate buffer, inject an 800 μL aliquot of the mixture onto column A and elute to waste with mobile phase A, after 4 min divert the effluent from column A onto column B. After 8 min elute the contents of column B onto column C with mobile phase B, after 1 min remove column B from the circuit, elute column C with mobile phase B, monitor the effluent from column C. (Flush column B with MeOH at 0.8 mL/min when not in line with column A.)

HPLC VARIABLES

Column: A 100 × 10 Sephadex G-25 superfine Pharmacia HR 10/10 column (Pharmacia Biotech, UK); B 10 × 2.1 37.5 μm Whatman pellicular ODS; C 10 × 3.2 5 μm Spherisorb ODS2 + 250 × 4.6 5 μm Spherisorb ODS2
Mobile phase: A 20 mM pH 6.5 ammonium phosphate buffer; B MeCN:buffer 42:58 (Buffer was 100 mM tetramethylethylenediamine solution adjusted to pH 7.0 with 85% phosphoric acid.)
Flow rate: A 1; B 0.8
Detector: UV 255

CHROMATOGRAM

Retention time: 11.51
Internal standard: (αR,βS)-α-(2-chlorophenyl)-5-fluoro-β-methyl-α-(1H-1,2,4-triazol-1-ylmethyl)-4-pyrimidineethanol (UK-115 794) (13.55)
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES

Noninterfering: acetaminophen, didanosine, prednisolone, rifampicin, zidovudine

KEY WORDS

column-switching; plasma; SEC

REFERENCE
Stopher,D.A.; Gage,R. Determination of a new antifungal agent, voriconazole, by multidimensional high-performance liquid chromatography with direct plasma injection onto a size-exclusion column, *J.Chromatogr.B*, **1997**, *691*, 441–448.